Research Article

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Nigella sativa L. as an alternative antibiotic feed supplement and effect on growth performance in weanling pigs

Branko T Petrujkić,[a] Ross C Beier,[b*] Haiqi He,[b] Kenneth J Genovese,[b] Christina L Swaggerty,[b] Michael E Hume,[b] Tawni L Crippen,[b] Roger B Harvey,[b] Robin C Anderson[b] and David J Nisbet[b]

Abstract

BACKGROUND: *Nigella sativa* L. (*NS*) is a plant containing bioactive constituents such as thymoquinone. Extracts of *NS* improve performance and reduce enteropathogen colonization in poultry and small ruminants, but studies with swine are lacking. In two different studies oral administration of *NS* extracts at doses equivalent to 0, 1.5 and 4.5 g kg^{-1} diet was assessed on piglet performance and intestinal carriage of wildtype *Escherichia coli* and *Campylobacter*, and *Salmonella* Typhimurium.

RESULTS: Wildtype *E. coli* populations in the jejunal and rectal content collected 9 days after treatment began were decreased ($P \leq 0.05$). Populations recovered from pigs treated with extract at 1.5 and 4.5 g kg^{-1} diet were 0.72–1.31 log$_{10}$ units lower than the controls (ranging from 6.05 to 6.61 log$_{10}$ CFU g^{-1}). Wildtype *Campylobacter* and *Salmonella* Typhimurium were unaffected by *NS* treatment. Feed efficiency over the 9 days improved linearly ($P < 0.05$) from 3.88 with 0 *NS*-treated pigs to 1.47 and 1.41 with pigs treated with *NS* at 1.5 and 4.5 g kg^{-1} diet, respectively, possibly due to high glutamine/glutamic acid content of the *NS* extract.

CONCLUSION: *NS* supplementation of weanling pigs improved feed efficiency and helped control intestinal *E. coli* during this vulnerable production phase.

Keywords: alternative antibiotic; *Escherichia coli*; feed conversion ratio; feed supplement; swine; weanling pigs

INTRODUCTION

Livestock producers are under increasing pressure to reduce their use of antibiotics to control disease and enhance production. This pressure is due to the concern of public health officials and the general public that such use may contribute to the emergence and proliferation of microbial populations resistant to antibiotics used to treat medically important diseases. It is not known, however, what will happen to levels of important zoonotic pathogens on the farm and in the processing plant should usage of currently available antibiotics be discontinued. Consequently, producers as well as consumers share a critical need for new, environmentally compatible technologies to aid in growth promotion and help eliminate pathogens from food animals during on-farm rearing. In particular, a new and effective environmentally compatible technology to underpin the problem of early weaning stress in pigs would be advantageous. The innate mucosal immune responses to enterotoxigenic *Escherichia coli* has been shown to be impaired by early weaning stress in pigs, and this stress also exacerbates intestinal injury and clinical disease.[1]

A particularly attractive plant source has been identified that contains antimicrobial activity called *Nigella sativa* L. (*NS*).[2] This plant is native to Asia, parts of the Middle east and Southwest Asia, and has been used for thousands of years to preserve spices and food,[2–4] and medicinally throughout the world.[5] The seeds have been used as traditional medicine for centuries for treatment of different ailments, including diabetes, hypertension and cardiac disease.[6] *NS* is known to contain a variety of different thymol-type derivatives such as thymoquinone (Fig. 1), dithymoquinone and thymodihydroquinone, as well as larger carbonyl polymers such as nigellone.[7,8] But thymoquinone is considered one of the major bioactive components in *NS*,[9] and comprises approximately 360 g kg^{-1} of the *NS* essential oil.[2] The plant's seeds (sometimes called black cumin seeds) also contain up to 300 g kg^{-1} fat with nearly 180 g kg^{-1} of the fat being the highly nutritious fatty acid linoleic acid.[7] The seeds also contain a variety of other biologically active compounds such as alkaloids, saponins and tannins.[7] *NS*, whether fed to poultry or goats as whole seeds or extracts,

* *Correspondence to: RC Beier, Food and Feed Safety Research Unit, Southern Plains Agricultural Research Center, Agricultural Research Service, USDA, College Station, TX 77845, USA. E-mail: ross.beier@ars.usda.gov*

a *Department of Nutrition and Botany, Faculty of Veterinary Medicine, Belgrade University, Belgrade, Serbia*

b *Food and Feed Safety Research Unit, Southern Plains Agricultural Research Center, ARS, USDA, College Station, TX, USA*

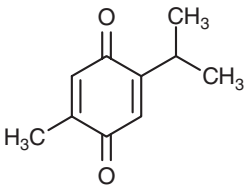

Figure 1. Structure of thymoquinone.

has been shown to be biologically active, exhibiting antimicrobial, anti-inflammatory, antioxidant, gastro-protective and anticancer benefits, as well as improved egg production and shell quality, along with other activities.[7,8,10,11]

The antimicrobial, anti-inflammatory and gastro-protective activities of *NS* seeds or extracts have been demonstrated *in vitro*[12] and extensively in poultry with several log-fold reductions of ileal or excreta concentrations of *E. coli* in laying hens and broilers,[13,14] and in Baladi goat kids in Egypt also improving immune function.[15] These findings provide clear evidence that the biological activity of *NS* can pass through the digestive tract and be active in the lower gastrointestinal area. It has been shown that thymoquinone completely inhibits *E. coli* ATP synthase.[16] This inhibition links the beneficial dietary based antimicrobial and the anticancer effects to thymoquinone.[16] Significant improvement in animal performance measures have been observed, with 19–33% improvement in feed conversion observed in laying hens,[11,17] and approximately 3–5% improvement in feed conversion observed in broilers.[18,19] Additionally, improved carcass characteristics and decreased illness and death have been consistently observed for poultry, rabbits and small ruminants.[20]

To date, little research, if any, has been conducted with *NS* seeds or extracts in swine, probably because most of the work has been limited to the geographical regions where little or no swine production exists. The objectives of this work were to test an *NS* extract for use as an alternative antibiotic feed supplement for its effects on wildtype populations of *E. coli* and *Campylobacter* and a challenge strain of *Salmonella* Typhimurium and also evaluate its effect on growth performance in weanling pigs.

MATERIALS AND METHODS

Animals

The care and handling procedures used for animals during this research were conducted according to procedures approved by the USDA/ARS Southern Plains Area Institutional Animal Care and Use Committee protocol #2016016.

A total of 72 3-week-old weaned Yorkshire × American Landrace crossbreed piglets were obtained and acclimated from 2 to 5 days at the USDA Food and Feed Safety Research Unit, College Station, TX, USA. The weanling piglets were then randomly placed in rearing pens (two pigs per pen to allow for social contact). Based on a report describing a diet that contained 170 g kg^{-1} crude protein provided better growth performance and decreased the incidence of diarrhea for 21- to 35-day-old weaned piglets compared to higher protein levels,[21] all animals in our study were fed a diet consisting of 170 g kg^{-1} protein, 20 g kg^{-1} fat, 20 g kg^{-1} fiber and 12 g kg^{-1} lysine. Fiber content is appropriate when kept between 15 and 30 g kg^{-1}.[22,23] Animals had unrestricted access to drinking water via nipple drinkers, while the feed was provided twice daily (07:00 and 14:00 h) *ad libitum* over the course of the experiments. The animals were divided randomly into two study groups, $n = 36$ per study group. Each study group contained a control 0× ($n = 12$), a 1× ($n = 12$) and a 3× ($n = 12$) treatment. The 0× group received no added *NS* extract, the 1× group received *NS* extract at 1.5 g kg^{-1} diet matter and the 3× group received *NS* extract at 4.5 g kg^{-1} diet matter. Each study group consisted of six replicated pens with two piglets in each pen (for a total of 36 piglets and 18 pens). The *NS* extract was given by oral gavage in Study 1, and it was incorporated in the feed for Study 2. The feed intake per pen was recorded daily in Study 2, while the body weights were recorded at the beginning and end of the study to evaluate animal growth and the feed conversion ratio (FCR).

Table 1. Amino acid analysis data for the *Nigella sativa* seed meal extract

Amino acid	Molar composition of total protein[a] (%)	Weight composition of total protein[a] (g kg^{-1})
Alanine	5.7	4.92
Arginine	6.3	11.7
Asparagine/aspartic acid	9.3	12.76
Glutamine/glutamic acid	32.7	50.24
Glycine	11.8	7.97
Histidine	1.5	2.39
Isoleucine	2.7	3.59
Leucine	4.0	5.45
Lysine	2.8	4.25
Methionine	1.6	2.39
Phenylalanine	1.8	3.19
Proline	5.0	5.72
Serine	5.3	5.58
Threonine	3.9	4.65
Tyrosine	1.6	3.06
Valine	4.1	4.92
Total		132.78

[a] Total protein in the *Nigella sativa* extract = 132.9 g kg^{-1}.

NS extract

Extraction of *NS* seed meal was performed similar to that reported by Yasmeen and Hassnain.[24] Briefly, the overnight percolation-assisted extraction method was used with 50% ethanol, and then concentrated by rotoevaporation at 55 °C for 12 h and further dried at 50 °C for 18 h, and the material was then stored in a cool dry place until used. For Study 2 the feed ration and *NS* extract were mixed at the Texas A&M University Feed Mill, College Station, TX, USA.

Amino acid analysis

The amino acid analysis of the *NS* extract was performed at the Texas A&M University Protein Chemistry Laboratory, College Station, TX, USA.

Salmonella Typhimurium challenge strain

Qualitative culture[25] of rectal swabs collected from the pigs upon arrival to the Food and Feed Safety Research Unit facilities revealed that only 10 of the 72 pigs were culture-positive for wildtype

Table 2. Effect of various concentrations (0, 1.5 and 4.5 g) of *Nigella sativa* extract kg^{-1} given by oral gavage to weanling pigs at 6 h and 18 h following oral gavage with NN-resistant *Salmonella* Typhimurium on wildtype *E. coli* and *Campylobacter* species and the experimentally inoculated NN-resistant *Salmonella* Typhimurium at 26 h following oral gavage of NN-resistant *Salmonella* Typhimurium (Study 1)

	Nigella sativa extract					
	0	1.5	4.5 g kg^{-1}	Polynomial contrasts		
Bacteria (gut location)	($\log_{10}$ CFU g^{-1})			Linear	Quadratic	SEM
Wildtype *Escherichia coli*						
Jejunal	5.84	4.95	4.60	0.1854	0.5272	0.590
Cecal	7.58	7.50	6.98	0.2773	0.8151	0.401
Rectal	6.83	6.58	6.78	0.9989	0.6124	0.395
Wildtype *Campylobacter* spp.						
Jejunal	2.17	2.22	2.22	0.8749	0.8692	0.162
Cecal	4.72	4.55	4.49	0.7620	0.8841	0.499
Rectal	5.23	5.01	4.96	0.7152	0.8210	0.459
NN-resistant *Salmonella* Typhimurium						
Jejunal	1.05	1.14	1.40	0.6507	0.9776	0.531
Cecal	3.61	2.92	3.98	0.4246	0.1844	0.469
Rectal	3.08	2.84	3.37	0.7985	0.2285	0.424

Table 3. Effect of various concentrations (0, 1.5 and 4.5 g) of *Nigella sativa* extract kg^{-1} given by oral gavage to weanling pigs at 6 h and 18 h following oral gavage with NN-resistant *Salmonella* Typhimurium on wildtype *E. coli* and *Campylobacter* species and the experimentally inoculated NN-resistant *Salmonella* Typhimurium at 42 h following oral gavage of NN-resistant *Salmonella* Typhimurium (Study 1)

	Nigella sativa extract					
	0	1.5	4.5 g kg^{-1}	Polynomial contrasts		
Bacteria (gut location)	($\log_{10}$ CFU g^{-1})			Linear	Quadratic	SEM
Wildtype *Escherichia coli*						
Jejunal	6.25a	5.59ab	4.44b	0.0263	0.9284	0.569
Cecal	8.17	7.65	7.18	0.0938	0.6997	0.383
Rectal	7.77	6.69	6.64	0.0936	0.1749	0.395
Wildtype *Campylobacter* spp.						
Jejunal	2.61	2.05	2.03	0.4214	0.5223	0.436
Cecal	5.17	5.28	4.76	0.4679	0.6710	0.448
Rectal	5.01	4.94	5.02	0.9699	0.8909	0.415
NN-resistant *Salmonella* Typhimurium						
Jejunal	ND	ND	ND	–	–	–
Cecal	3.29	4.07	3.53	0.9009	0.2922	0.510
Rectal	3.02	2.85	3.12	0.8388	0.7345	0.478

A general ANOVA revealed a tendency for main effect of treatment ($P = 0.0770$), with means followed by unlike letters (a, b) differing at $P < 0.05$ based on a least significant difference multiple comparison of means. ND, not detected.

Salmonella. Accordingly, all pigs were experimentally challenged with a *S. enterica* serovar Typhimurium (NVSL 95–1776) strain to homogeneously distribute *Salmonella* to the experimental populations. The challenge strain possessed natural resistance to novobiocin at 25 μg mL^{-1} (Sigma-Aldrich, St Louis, MO, USA) and was previously made resistant to nalidixic acid (Sigma-Aldrich, St Louis, MO, USA) via successive cultivations in tryptic soy broth (TSB) (Becton, Dickinson & Co., Sparks, MD, USA) containing up to 20 μg mL^{-1} nalidixic acid.[26] A 24 h old culture of this novobiocin and nalidixic acid (NN)-resistant *Salmonella* Typhimurium grown in TSB to a concentration of 2.25×10^8 CFU mL^{-1} was used to challenge the weanling pigs in Study 1, and a second growth medium with an NN-resistant *Salmonella* Typhimurium concentration of 2.0×10^9 CFU mL^{-1} was used to challenge the weanling pigs in Study 2.

Study 1

In this study the *NS* extract was given via oral gavage to 26-day-old weanling pigs at 6 and 18 h following the NN-resistant *Salmonella* Typhimurium oral gavage challenge of 6 mL growth medium for a total of 1.35×10^9 viable cells. The control group was gavaged with 6 mL distilled water, while the 1.5 g kg^{-1} *NS* extract group was gavaged with 6 mL of a mixture of *NS* extract at 100 g kg^{-1} water and the 4.5 g kg^{-1} *NS* extract group received 6 mL of a mixture of *NS* extract at 300 g kg^{-1} water, which was equivalent to 0.6 and 1.8 g dry *NS* extract per animal, respectively. One pig per pen was euthanized and necropsied at 26 h ($n = 18$) and the second pig was euthanized and necropsied at 42 h ($n = 18$) following the NN-resistant *Salmonella* Typhimurium oral gavage challenge, and were subjected to microbiology analyses.

Study 2

In Study 2 the *NS* extract was provided in the feed to 23-day-old weanling piglets from the first day of the study. The 1.5 and 4.5 g kg^{-1} *NS* extract treatments were mixed with the diet, and the 0, 1.5 and 4.5 g kg^{-1} *NS* extract treatments were offered for each meal. Animals were orally challenged with 6 mL of the second growth medium for a total of 1.2×10^{10} viable cells of NN-resistant *Salmonella* Typhimurium on the 7th day of treatment. Pigs were euthanized and necropsied 42 h following the oral challenge. Feed offered over the duration of the experiment (9 days) and not consumed was collected daily, and dried and weighed to determine the daily dry matter feed consumption.

Microbiological sampling

Gut samples from the jejunum, cecum and rectum were collected at necropsy and serially diluted in phosphate-buffered saline (pH 6.5) and plated on Campy Cefex Agar as prepared in previous studies,[27,28] Brilliant Green Agar (Oxoid Ltd, Basingstoke, UK) and 3 M *E. coli*/coliform petrifilm (3 M Health Care, St Paul, MN, USA) to enumerate *Campylobacter*, *Salmonella* and *E. coli*, respectively. The Brilliant Green Agar was supplemented with 25 μg novobiocin mL^{-1} and 20 μg nalidixic acid mL^{-1} to facilitate enumeration of the NN-resistant *Salmonella* Typhimurium challenge organism. *Campylobacter* organisms were enumerated after 48 h of microaerophilic (N_2–CO_2–O_2; 85:10:5) incubation at 42 °C. NN-resistant *Salmonella* Typhimurium and wildtype *E. coli* were counted after 24 h aerobic incubation at 37 °C. Viable cell counts of wildtype *Campylobacter* and *E. coli*, and NN-resistant *Salmonella* Typhimurium were log transformed and expressed as concentrations ($\log_{10}$ CFU g^{-1} contents).

Statistics

Bacterial concentrations determined after each necropsy in both Studies 1 and 2 were analyzed separately by an analysis of variance (ANOVA). Dry matter intake, weight gain, FCR and bacterial concentrations were tested for treatment effects using a general analysis of variance. Polynomial contrasts were used to examine linear and quadratic effects of treatment level. Where the ANOVA revealed a significant main effect of treatment or a tendency to such, a least significant difference test was used to separate treatment means. Non-significant main effects ($P > 0.10$) resulting from the analysis of variance are not presented. All procedures were completed using Statistix version 10 (Tallahassee, FL, USA) and a 'pen' was considered the experimental unit.

RESULTS

Amino acid analysis

The average amount of total protein in the *NS* extract was 132.9 g kg^{-1}. Table 1 shows the amino acid content of the *NS* extract by molar composition and by weight composition. The glutamine/glutamic acid amino acid composite was the greatest of all the amino acids in the protein fraction of the *NS* extract, with a weight composition of 50.24 g kg^{-1}.

Study 1

Tables 2 and 3 present the data at 26 and 42 h following oral gavage of NN-resistant *Salmonella* Typhimurium, respectively, for all bacteria evaluated in the jejunal, cecal and rectal content of the weaned pigs with respect to the amount of *NS* extract provided by gavage: 0, 1.5 and 4.5 g kg^{-1} diet. Gut concentrations of wildtype *E. coli* were not significantly affected by the *NS* extract treatment at 26 h (Table 2), but *E. coli* were significantly decreased in the jejunal content with linear polynomial contrasts at $P = 0.0263$; whereas a general ANOVA revealed only a tendency for a main effect of treatment ($P = 0.0770$) with treatment means at $P < 0.05$ based on a least significant difference multiple comparison of means at 42 h post-oral gavage with the *NS* extract (Table 3). Numbers of wildtype *Campylobacter* spp. and the NN-resistant *Salmonella* Typhimurium challenge strain were unaffected by oral gavage with all concentrations of the *NS* extract.

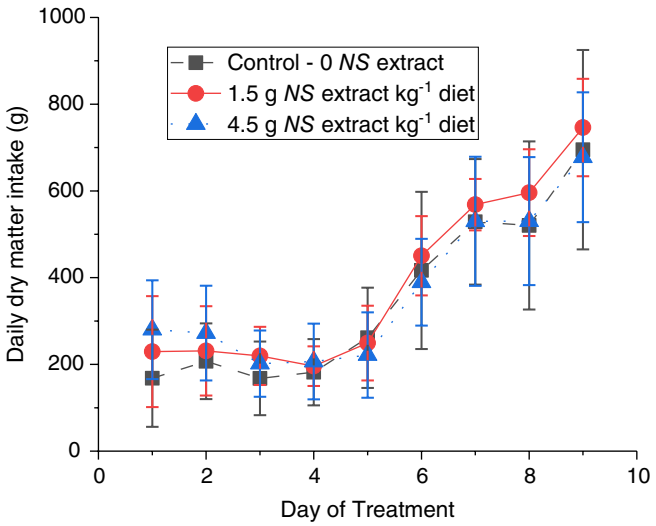

Figure 2. Daily dry diet matter intake per pig (Study 2). Values are mean ± SD from $n = 6$ replicated pens (2 pigs per pen and 12 pigs per treatment).

Study 2

Figure 2 shows the daily dry matter intake of the control group, and of the 1.5 and 4.5 g kg^{-1} *NS* extract fed groups of weanling pigs. There were no differences in the intake of dry matter between the three different treatment groups (Fig. 2), with pigs consuming 33–71% of feed offered daily.

Table 4 shows the effects of feeding the *NS* extract on wildtype *E. coli* and *Campylobacter* spp. and inoculated NN-resistant *Salmonella* Typhimurium 42 h after challenge with NN-resistant *Salmonella* Typhimurium. Numbers of wildtype *Campylobacter* spp. and the NN-resistant *Salmonella* Typhimurium challenge strain were unaffected by treatment with the *NS* extract. The *NS* extract treatment demonstrated a significant decreasing ($P < 0.05$) linear effect on wildtype *E. coli* in the jejunal content and in the rectal content. There also was a significant quadratic effect on wildtype *E. coli* in the jejunal content.

Table 5 shows the average total feed consumed per pen over the duration of Study 2. The feed intake for the 0, 1.5 and 4.5 g kg^{-1} *NS* extract treatment groups averaged 3.65 ± 0.19 kg per pen. The average total gain per pen was also unaffected by treatment. However, the average FCR revealed significant linear effects ($P = 0.0493$) of treatment, resulting in a 62.1% and 63.7% improved FCR ($P < 0.05$) for the 1.5 and 4.5 g kg^{-1} *NS* extract treatments over the 0 *NS* extract control treatment, respectively.

Table 4. Effect of feeding various concentrations (0, 1.5 and 4.5 g) of *Nigella sativa* extract kg^{-1} diet to weanling pigs for 9 days on wildtype *E. coli* and *Campylobacter* species and the experimentally inoculated NN-resistant *Salmonella* Typhimurium at 42 h after challenge with NN-resistant *Salmonella* Typhimurium (Study 2)

	Nigella sativa extract			Polynomial contrasts		
	0	1.5	4.5 g kg^{-1}			
Bacteria (gut location)	($\log_{10}$ CFU g^{-1})			Linear	Quadratic	SEM
Wildtype *Escherichia coli*						
Jejunal	6.05a	4.48b	4.74b	0.0237	0.0083	0.324
Cecal	6.50	5.66	5.60	0.1318	0.2423	0.364
Rectal	6.61c	5.89d	5.68d	0.0188	0.1868	0.246
Wildtype *Campylobacter* spp.						
Jejunal	2.04	1.55	2.05	0.8009	0.2296	0.323
Cecal	4.04	3.40	3.53	0.3675	0.2527	0.324
Rectal	4.42	4.59	4.17	0.5199	0.5627	0.344
NN-resistant *Salmonella* Typhimurium						
Jejunal	1.25	1.00	1.84	0.1238	0.2597	0.312
Cecal	2.34	2.10	2.91	0.2769	0.4254	0.428
Rectal	2.84	2.42	2.55	0.6754	0.4892	0.374

A general ANOVA revealed a main effect of treatment ($P = 0.0032$) with means followed by unlike letters (a, b) differing at $P < 0.05$ based on a least significant difference multiple comparison of means.
A general ANOVA revealed a main effect of treatment ($P = 0.0281$) with means followed by unlike letters (c, d) differing at $P < 0.05$ based on a least significant difference multiple comparison of means.

Table 5. Effect of feeding various concentrations (0, 1.5 and 4.5 g) of *Nigella sativa* extract kg^{-1} diet to weanling pigs for 9 days on total amount of feed consumed, total weight and average daily gain revealing a significant effect of treatment on the feed conversion ratio (Study 2)

Nigella sativa extract			Polynomial contrasts		
0	1.5	4.5 g kg^{-1}	Linear	Quadratic	SEM
Average total food consumed per pen (g)					
3462	3835	3641	0.8284	0.5011	364.2
Average total gain per pen (g)					
1840	2745	3062	0.1705	0.4852	558.0
Average daily gain per pen (g)					
204	305	340	0.1720	0.4845	62.0
Average feed conversion ratio per pen (intake/gain)					
3.88a	1.47b	1.41b	0.0493	0.0954	0.715
Change in feed conversion ratio from the 0 *Nigella sativa* extract treatment (%)					
0.0	62.1%	63.7%			

A general ANOVA revealed a main effect of treatment ($P = 0.0441$) with means within rows followed by unlike letters (a, b) differing at $P < 0.05$, based on a least significant difference multiple comparison of means.

DISCUSSION

Amino acid analysis of the *NS* extract conducted in this study demonstrated a reasonable amount of 50% methanol-soluble protein, which was good since in previous investigations the protein content of black cumin seeds from six different sources was evaluated between 199 and 241 g kg^{-1} protein.[29,30] Our finding that the *NS* treatment exhibited anti-*E. coli* activity in treated swine supports earlier reports demonstrating an anti-*E. coli* effect of *NS* components when tested *in vitro*[12] or after administration to poultry and small ruminants.[13–15] To our knowledge, our finding that treatment of swine with the *NS* extract had no effect on gut concentrations of wildtype *Campylobacter* spp. has not been previously reported. *Campylobacter* spp. are known to be less sensitive to free thymol than *E. coli* or Salmonella,[28] and thus it is reasonable to suspect that *Campylobacter* may likewise be less sensitive to the thymol derivatives contained within the *NS* extract. Our finding that *NS* treatment had no effect on the NN-resistant *Salmonella* Typhimurium challenge strain is not in agreement with the results of Kumar *et al.*,[31] who reported decreases in cecal *Salmonella* concentrations in broilers fed ground black cumin seed. However, these researchers observed no effect of black cumin seed on gut concentrations of *E. coli*, or *Lactobacillus* and *Clostridium* spp.[31] Comparisons between these studies must be made cautiously, however, as differences in study design and in how the *NS* seeds or extracts were prepared and administered likely contribute greatly to the differential findings. Mechanistically, at least one of the components of the *NS* extract – thymoquinone – has been reported to inhibit ATP

synthase activity of *E. coli*,[16] but other potential mechanisms cannot be excluded.

Results from this study indicate the *NS* extract treatment increased FCR efficiency in the piglets, which is consistent with earlier reports demonstrating that *NS* seeds and extracts improved FCR efficiency in poultry.[11,17–20] Kumar and colleagues had reported improved nutrient metabolizability in broilers fed ground *NS* seed.[31] The *NS* extract used in the present study contained amino acids – some at a relatively high concentration (Table 1) – that would not have been available to the untreated pigs, as no attempt was made to keep amino acid profiles similar between treated and control pigs. Based on measured intakes of pigs fed *NS* extract at 1.5–4.5 g kg^{-1} diet dry matter, they would have consumed a total of approximately 0.38–1.09 g total protein that was not available to the control pigs. In the case of the most abundant amino acid – glutamine/glutamic acid – this alone would have provided 0.14 and 0.41 g to each pig fed *NS* extract at 1.5 and 4.5 g kg^{-1} diet dry matter over the whole study period of 9 days, respectively. Since this is a relatively low concentration of glutamine/glutamic acid, we did not adjust the amino acid concentration in the diet of the untreated control pigs to compensate for the amino acids present in the *NS* extract. Whether this increased intake of glutamine/glutamic acid by the treated pigs is a contributing factor affecting the feed conversion in this study needs to be elucidated in further research. Although glutamine is considered a conditionally essential amino acid, it is utilized as a significant fuel source by mucosal leukocytes – in particular, lymphocytes – and by small intestinal epithelial cells.[32] Glutamine helps restore intestinal tight junction integrity,[33] and improves intestinal barrier function in rats[34,35] and in piglets.[36,37] Glutamine is also the dominant nitrogen source for purine synthesis, and the requirement is dependent on the mitotic rate within the normal mucosa. In short-term rat and pig studies, adding glutamine to nutritional intravenous solutions reduced some aspects of disuse intestinal atrophy and enhanced intestinal immune function.[38] With this in mind, and knowing that piglets are subjected to stress during the weaning period, their requirements for glutamine may be higher and its supplementation may be beneficial. Several animal studies have demonstrated that enteral glutamine supplementation enhances gut mucosal growth and repair, decreases bacterial translocation and inflammation, and improves nitrogen balance in animal models of intestinal atrophy, injury and adaptation.[39,40] The observed difference in the studies was attributed to differences in utilization of glutamine by enterocytes or leukocytes, or to differences in the intestinal hormone-dependent trophic response to whole proteins rather than to elemental diets.[40]

CONCLUSIONS

Results from the present work extend earlier findings by demonstrating that oral administration of an *NS* extract to weanling pigs improved feed conversion by 63.7% in the 4.5 g kg^{-1} *NS* extract treatment group over the controls during the 9-day trial and significantly reduced levels of naturally occurring *E. coli* in the jejunal and rectal content. The improvement in FCR efficiency in the present study was achieved due to modest, nonsignificant effects of feed intake in conjunction with nonsignificant body weight gain, the latter due possibly to epithelial utilization of the glutamic acid/glutamine content of the *NS* extract. These results warrant further research to investigate the role that glutamine/glutamic acid may play in *NS* supplementation and the potential of *NS* to control specific enteropathogenic *E. coli* infections in weanling pigs and to improve pig performance during grower and finisher phases of pig production.

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REFERENCES

1 McLamb BL, Gibson AJ, Overman EL, Stahl C and Moeser AJ, Early weaning stress in pigs impairs innate mucosal immune responses to enterotoxigenic *E. coli* challenge and exacerbates intestinal injury and clinical disease. *PLoS ONE* **8**:e59838 (2013).
2 Venkatachallam SKT, Pattekhan H, Divakar S and Kadimi US, Chemical composition of *Nigella sativa* L. seed extracts obtained by supercritical carbon dioxide. *J Food Sci Technol* **47**:598–605 (2010).
3 Ismail M, Al-Naqeep G and Chan KW, *Nigella sativa* thymoquinone-rich fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats. *Free Radic Biol Med* **48**:664–672 (2010).
4 Machmudah S, Shiramizu Y, Goto M, Sasaki M and Hirose T, Extraction of *Nigella sativa* L. using supercritical CO_2: a study of antioxidant activity of the extract. *Sep Sci Technol* **40**:1267–1275 (2005).
5 Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA *et al.*, A review on therapeutic potential of *Nigella sativa*: a miracle herb. *Asian Pac J Trop Biomed* **3**:337–352 (2013).
6 Hadi MY, Mohammed GJ and Hameed IH, Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography–mass spectrometry. *J Pharmacogn Phytother* **8**:8–24 (2016).
7 Khan MA, Chemical composition and medicinal properties of *Nigella sativa* Linn. *Inflammopharmacology* **7**:15–35 (1999).
8 Paarakh PM, *Nigella sativa* Linn. – a comprehensive review. *Indian J Nat Prod Res* **1**:409–429 (2010).
9 Salea R, Widjojokusumo E, Hartanti AW, Veriansyah B and Tjandrawinata RR, Supercritical fluid carbon dioxide extraction of *Nigella sativa* (black cumin) seeds using Taguchi method and full factorial design. *Biochem Compd* **1**:1 https://doi.org/10.7243/2052-9341-1-1 (2013).
10 Salmani JMM, Asghar S, Lv H and Zhou J, Aqueous solubility and degradation kinetics of the phytochemical anticancer thymoquinone; probing the effects of solvents, pH and light. *Molecules* **19**:5925–5939 (2014).
11 Khan SH, Anjum MA, Parveen A, Khawaja T and Ashraf NM, Effects of black cumin seed (*Nigella sativa* L.) on performance and immune system in newly evolved crossbred laying hens. *Vet Q* **33**:13–19 (2013).
12 Hanafy MSM and Hatem ME, Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). *J Ethnopharmacol* **34**:275–278 (1991).
13 Boka J, Mahdavi AH, Samie AH and Jahanian R, Effect of different levels of black cumin (*Nigella sativa* L.) on performance, intestinal *Escherichia coli* colonization and jejunal morphology in laying hens. *Anim Physiol Anim Nutr* **98**:373–383 (2014).

14 Bölükbaşi ŞC, Kaynar Ö, Erhan MK and Ürüþan H, Effect of feeding *Nigella sativa* oil on laying hen performance, cholesterol and some proteins ration of egg yolk and *Escherichia coli* count in feces. *Arch Geflügelkde* **73**:167–172 (2009).
15 Abou-Zeina HAA, Ghazy AA, El-Bayoumy MK, Dorgham SM, Khairy EA and Twfik HI, Effects of dietary antioxidants supplementation on cellular immune response and evaluation of their antimicrobial activity against some enteric pathogens in goats. *Global Vet* **11**:145–154 (2013).
16 Ahmad Z, Laughlin TF and Kady IO, Thymoquinone inhibits *Escherichia coli* ATP synthase and cell growth. *PLoS ONE* **10**:e0127802 (2015).
17 Hassan SM and Alaqil AA, Effect of feeding different dietary levels of black cumin (*Nigella sativa* L.) seed on productive performance in laying hens. *Asian J Poult Sci* **8**:41–48 (2014).
18 Guler T, Dalkihç B, Ertas ON and Cifçi M, The effect of dietary black cumin seeds (*Nigella sativa* L.) on the performance of broilers. *Asian–Aust J Anim Sci* **3**:425–430 (2006).
19 Siddiqui MN, Islam MT, Sayed MA and Hossain MA, Effect of dietary supplementation of acetone extracts of *Nigella sativa* L. seeds on serum cholesterol and pathogenic intestinal bacterial counts in broilers. *J Anim Plant Sci* **25**:372–379 (2015).
20 Longato E, Meineri G and Peiretti PG, Nutritional and zootechnical aspects of *Nigella sativa*: a review. *J Anim Plant Sci* **25**:921–934 (2015).
21 Wu Y, Jiang Z, Zheng C, Wang L, Zhu C, Yang X *et al.*, Effects of protein sources and levels in antibiotic-free diets on diarrhea, intestinal morphology, and expression of tight junctions in weaned piglets. *Anim Nutr* **1**:170–176 (2015).
22 Pascoal LAF, Thomaz MC, Watanabe PH, Ruiz UdS, Ezequiel JMB, Amorim AB *et al.*, Fiber sources in diets for newly weaned piglets. *R Bras Zootec* **41**:636–642 (2012).
23 Hubbard Feeds, First course early-weaning prestarter. [Online]. Available: http://www.hubbardfeeds.com/product/first-course-early-weaning-prestarter-2 [8 August 2017].
24 Yasmeen H and Hassnain S, Comparative analysis of different bioactivities of *Curcuma longa*, *Nigella sativa* seeds, and *Camellia sinensis* extracted by four different methods: a green way to reduce oxidative stress. *Food Sci Biotechnol* **25**:811–819 (2016).
25 Anderson RC, Stanker LH, Young CR, Buckley SA, Genovese KJ, Harvey RB *et al.*, Effect of competitive exclusion treatment on colonization of early-weaned pigs by *Salmonella* serovar Choleraesuis. *Swine Health Prod* **7**:155–160 (1999).
26 Anderson RC, Jung YS, Oliver CE, Horrocks SM, Genovese KJ, Harvey RB *et al.*, Effects of nitrate or nitro supplementation, with or without added chlorate, on *Salmonella enterica* serovar Typhimurium and *Escherichia coli* in swine feces. *J Food Protect* **70**:308–315 (2007).
27 Stern NJ, Wojton B and Kwiatek K, A differential-selective medium and dry ice-generated atmosphere for recovery of *Campylobacter jejuni*. *J Food Protect* **55**:514–517 (1992).
28 Anderson RC, Krueger NA, Byrd JA, Harvey RB, Callaway TR, Edrington TS *et al.*, Effects of thymol and diphenyliodonium chloride against *Campylobacter* spp. during pure and mixed culture *in vitro*. *J Appl Microbiol* **107**:1258–1268 (2009).
29 Babayan VK, Koottungal D and Halaby GA, Proximate analysis, fatty acid and amino acid composition of *Nigella sativa* L seeds. *J Food Sci* **43**:1314–1315, 1319 (1978).
30 Takruri HRH and Dameh MAF, Study of the nutritional value of black cumin seeds (*Nigella sativa* L). *J Sci Food Agric* **76**:404–410 (1998).
31 Kumar P, Patra AK, Mandal GP, Samanta I and Padhan S, Effect of black cumin seeds on growth performance, nutrient utilization, immunity, gut health and nitrogen excretion in broiler chickens. *J Sci Food Agric* **97**:3742–3751 (2017).
32 Ziegler TR, Young LS, Benfell K, Scheltinga M, Hortos K, Buy R *et al.*, Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition after bone marrow transplantation. A randomized, double-blind, controlled study. *Ann Intern Med* **116**:821–828 (1992).
33 Rao R and Samak G, Role of glutamine in protection of intestinal epithelial tight junctions. *J Epithel Biol Pharmacol* **5**(Suppl 1-M7):47–54 (2012).
34 Jiang J-W, Ren Z-G, Chen L-Y, Jiang L, Xie H-Y, Zhou L *et al.*, Enteral supplementation with glycyl-glutamine improves intestinal barrier function after liver transplantation in rats. *Hepatobiliary Pancreat Dis Int* **10**:380–385 (2011).
35 Shu X-L, Yu T-T, Zhong J-X and Lei T, Effect of glutamine on intestinal barrier function following liver transplantation in rats. *Eur Rev Med Pharmacol Sci* **18**:2058–2064 (2014).
36 Ewaschuk JB, Murdoch GK, Johnson IR, Madsen KL and Field CJ, Glutamine supplementation improves intestinal barrier function in a weaned piglet model of *Escherichia coli* infection. *Br J Nutr* **106**:870–877 (2011).
37 Wang B, Wu Z, Ji Y, Sun K, Dai Z and Wu G, L-Glutamine enhances tight junction integrity by activating CaMK kinase 2-AMP-activated protein kinase signaling in intestinal porcine epithelial cells. *J Nutr* **146**:501–508 (2016).
38 Remillard RL, Guerino F, Dudgeon DL and Yardley JH, Intravenous glutamine or limited enteral feedings in piglets: amelioration of small intestinal disuse atrophy. *J Nutr* **128**:2723S–2726S (1998).
39 Boza JJ, Maire J-C, Bovetto L and Ballèvre O, Plasma glutamine response to enteral administration of glutamine in human volunteers (free glutamine versus protein-bound glutamine). *Nutrition* **16**:1037–1042 (2000).
40 Preiser J-C, Peres-Bota D, Eisendrath P, Vincent J-L and Van Gossum A, Gut mucosal and plasma concentrations of glutamine: a comparison between two enriched enteral feeding solutions in critically ill patients. *Nutr J* **2**:13 (2003).

